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Dated 19 January 2000

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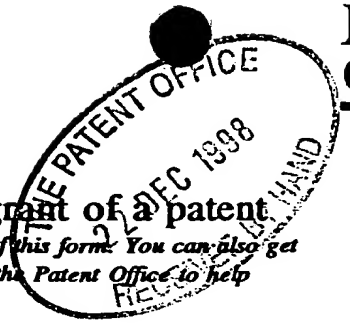

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The Patent Office

Cardiff Road
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1. Your reference — REP05972GB

2. Patent a — **9828359.1**
(The Patent Office will fill in this part) — 22 DEC 1998

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Microscience Ltd.
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

07304546001.

4. Title of the invention — PROTEIN AND COMPOSITIONS CONTAINING IT

5. Name of your agent *(if you have one)* — GILL JENNINGS & EVERY

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number *(if you know it)* — 745002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
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YES

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Continuation sheets of this form	
Description	6
Claim(s)	1
Abstract	
Drawing(s)	1+1-10

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Priority documents	
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature: Lucy Samuels

Date: 22 December 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

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PROTEIN AND COMPOSITIONS CONTAINING IT

Field of the Invention

This invention relates to one protein, to vaccines containing it, and to its use in therapy, for immunisation.

Background to the Invention

Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, is the causative agent of various conditions. In particular, GBS causes:

Early onset neonatal infection.

This infection usually begins *in utero* and causes severe septicaemia and pneumonia in infants, which is lethal if untreated and even with treatment is associated with a 10-20% mortality rate.

Late onset neonatal infection.

This infection occurs in the period shortly after birth until about 3 months of age. It causes a septicaemia, which is complicated by meningitis in 90% of cases. Other focal infections also occur including osteomyelitis, septic arthritis, abscesses and endopthalmitis.

Adult infections.

These appear to be increasingly common and occur most commonly in women who have just delivered a baby, the elderly and the immunocompromised. They are characterised by septicaemia and focal infections including osteomyelitis, septic arthritis, abscesses and endopthalmitis.

Urinary tract infections.

GBS is a cause of urinary tract infections and in pregnancy accounts for about 10% of all infections.

Veterinary infections.

GBS causes chronic mastitis in cows. This, in turn, leads to reduced milk production and is therefore of considerable economic importance.

GBS infections can be treated with antibiotics. However, immunisation is preferable. It is therefore

desirable to develop an immunogen that could be used in a therapeutically-effective vaccine.

Summary of the Invention

According to the present invention, a partial GBS gene sequence, pho1-13, has been found which represents the proteolytic subunit of an ATP-dependent CLP protease.

In one aspect of the invention, the use of this protein in a recombinant protein vaccine is described. This vaccine may be administered to females either prior to, or during pregnancy to protect mother and neonate against infection by GBS.

The gene sequence may be first genetically altered to increase the antigenicity of the encoded protein.

Brief Description of the Drawings

The invention will now be described in detail with reference to the accompanying figures, wherein:

Figure 1 shows the nucleotide sequence of the insert of clone pho1-13 and the deduced amino acid sequence of ORF1-13.

Description of the Invention

Because of its extracellular or cell surface location, the protein of the present invention may be a suitable candidate for the production of therapeutically-effective vaccines against GBS. The term "therapeutically-effective" is intended to include the prophylactic effect of the vaccines. For example, a recombinant protein may be used, as an antigen for direct administration to a patient. The protein may be isolated directly from GBS or expressed in any suitable expression system, e.g. *Lactococcus lactis*. It is preferably administered with an adjuvant, e.g. alum.

The protein may be a mutant protein, in comparison to wild-type protein, a fragment of the protein or a combination of different fragments, provided an effective immune response is generated.

An alternative approach is to use a live attenuated GBS vaccine. This may be produced by deleting the gene that encodes the protein. Preferably, the GBS strain

comprises additional virulence gene mutations.

The protein (or fragments thereof) of the present invention may also be used to produce monoclonal and polyclonal antibodies for use in passive immunisation.

In a further embodiment of the invention, the protein or corresponding polynucleotide may be used as a target for screening potentially useful drugs, especially antimicrobials. Suitable drugs may be selected for their ability to bind to the protein to exert their effects. Assays for screening for suitable drugs and which make use of the protein of the invention will be apparent to those skilled in the art.

Although the protein has been described for use in the treatment of patients, veterinary uses of the protein are also considered to be within the scope of the present invention. In particular, the protein or the vaccines may be used in the treatment of chronic mastitis, especially in cows.

The present invention is described with reference to Group B Streptococcal strain M732. However, all the GBS strains and many other bacterial strains are likely to include related proteins having amino acid sequence homology with the protein of M732. Organisms likely to contain the proteins include, but are not limited to, *S. pneumoniae*, *S. pyogenes*, *S. suis*, *S. milleri*, Group C and Group G *Streptococci* and *Enterococci*. Vaccines to each of these may be developed in the same way as described for GBS.

Preferably, the proteins that may be useful for the production of vaccines have greater than 40% sequence similarity with the protein of M732. More preferably, the proteins have greater than 60% sequence similarity. Most preferably, the proteins have greater than 80% sequence similarity.

The protein of the present invention was identified as follows:

A partial gene library of GBS (strain M732)

chromosomal DNA was prepared using the plasmid vectors pFW-*phoA*1, pFW-*phoA*2 and pFW-*phoA*3 (Podbielski, A. et al. 1996. Gene **177**:137-147). These plasmids possess a constitutive spectinomycin adenyltransferase antibiotic resistance marker, which confers a high level of spectinomycin resistance and is therefore easily selected. Furthermore, these vectors contain a truncated (leaderless) *Escherichia coli phoA* gene for alkaline phosphatase. The three vectors differ only with respect to the reading frame in which the leaderless *phoA* gene exists, as compared to an upstream in-frame *Bam*HI restriction enzyme site. Because this truncated *E. coli phoA* gene lacks the appropriate leader sequence for export of this enzyme across the bacterial membrane, extracellular alkaline phosphatase activity is absent when these plasmids are propagated in an *E. coli phoA* mutant (e.g. strain DH5α). The chromogenic alkaline phosphatase substrate, XP (5-Bromo-4-chloro-3-indolyl-phosphate), does not enter intact bacterial cells and therefore only exported or surface associated alkaline phosphatase activity can be detected. When exported or surface associated alkaline phosphatase activity is present, the chromogenic XP substrate is cleaved to yield a blue pigment and the corresponding bacterial colonies can be identified by their blue colour.

Plasmid DNA was digested to completion with *Bam*HI and dephosphorylated using shrimp alkaline phosphatase. GBS genomic DNA was partially digested with *Sau*3AI, size fractionated on a sucrose gradient and fragments <1kb in size were ligated into the prepared pFW-*phoA* vectors. *E. coli* strain DH5α was chosen as the cloning host since it lacks a functional *phoA* gene. Recombinant plasmids were selected on Luria agar containing 100 μg/ml of spectinomycin and 40 μg/ml of the chromogenic XP substrate. *E. coli* transformants harbouring plasmids containing GBS insert DNA that complements the export signal sequence of the leaderless *phoA* gene were identified by the blue colour of the colonies. Approximately 30000 different recombinant

plasmids containing GBS insert DNA were screened in this manner and 83 recombinant plasmids, which complemented the leaderless *phoA*, were chosen for further study.

From these experiments, one clone was selected containing a plasmid designated pho1-13. This plasmid contained a gene (or part thereof), which complemented the leaderless *phoA*. Plasmid pho1-13 contained 288 bp of GBS DNA and the nucleotide and deduced amino acid sequences are shown in Figure 1.

A comparison of the amino acid sequence of ORF1-13 was performed and the results are shown in Table 1.

As shown in Table 1, homologues to the GBS ORF1-13 gene product can be identified in *Streptococcus pyogenes*, *S. pneumoniae*, *S. salivarius*, *Escherichia coli*, *Yersinia enterocolitica*, *Aquifex aeolicus*, *Helicobacter pylori* and *Haemophilus influenzae*. The *S. pyogenes* and *S. pneumoniae* homologues were identified from genome sequence data and no annotations were available as to the identity of the gene or gene products. In all other cases, the above homologues can be identified as ATP-dependent CLP protease proteolytic subunits. The catalytic activity of Clp proteases results in the hydrolysis of proteins to small peptides in the presence of ATP and magnesium (Giffard, P.M. et al. 1993. J. Gen. Microbiol. **139**:913-920). Furthermore, the ClpP component of Clp proteases has been shown to be induced as part of the heat shock response (Kroh, H.E. and L.D. Simon. 1990. J. Bacteriol. **172**:6026-6034) and it is probable that this subunit or the complete proteolytic domain would associated with the bacterial surface.

Table 1. Database search results for ORF1-13 (96 amino acids)

Organism	Protein Accession	DNA Accession	Gene Name	% Similarity	% Identity	Alignment Length
S. salivarius	SW:P36398	EM:L07793	*clpP*	97.18	96.87	96
S. pyogenes	bp 15621-16208	Contig 266	Unknown	96.87	94.79	96
S. pneumoniae	bp 1984-2169	Contig 4322	Unknown	94.74	92.10	38
E. coli	SW:P19245	EM:J05534	*clpP* or *lopP*	73.96	63.54	96
Y. enterocolitica	SW:Q60107	EM:U55059	*clpP*	71.87	62.50	96
A. aeolicus	TR:O67357	EM:AE000735	*clpP*	71.87	61.46	96
H. pylori	SW:P56156	EM:AE000591	*clpP*	70.83	59.37	96
H. influenzae	SW:P43867	EM:U32754	*clpP*	70.83	58.33	96

CLAIMS

1. A surface-associated proteolytic protein obtainable from a Group B streptococcal strain.

2. A protein according to claim 1, obtainable from the Group B streptococcal strain M732.

3. A protein according to claim2, encoded by the polynucleotide defined as ORF1-13 in Figure 1 or a homologue thereof with at least 60% sequence homology.

4. A protein according to claim 3, wherein ORF1-13 comprises the nucleotides 1-288.

5. A protein according to any of claims 1 to 4, for use in a method of therapy.

6. A protein according to claim 5, for use in the treatment of GBS infection.

7. A polynucleotide which encodes a protein according to any preceding claim, its complement, or a fragment thereof.

8. The use of a bacterial protein according to any of claims 1 to 6, in the manufacture of a vaccine to treat bacterial infection.

9. The use according to claim 8, wherein the infection is a Group B streptococcal infection.

10. The use according to claim 8 or claim 9, wherein the infection is a focal infection.

11. The use according to claim 8 or claim 9, wherein the infection is a urinary tract infection.

12. Use of a product according to any of claims 1 to 7, for screening potential antimicrobial drugs.

13. An antimicrobial drug selected using the products as defined in claim 12.

14. A vaccine comprising a protein according to any of claims 1 to 6 and 8.

15. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene codes for a protein according to claim 8.

16. An antibody raised against a protein according to any of claims 1 to 4.

Figure 1. Nucleotide and deduced amino acid sequence of clone pho1-13

START ORF1-13 (truncated)

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|       10                  30                  50
GATCGTATTATTATGTTGACAGGCCAAGTTGAGGATAATATGGCCAATAG
D  R  I  I  M  L  T  G  Q  V  E  D  N  M  A  N  S

                    70                  90
TATCATTGCACAGTTATTGTTTCTCGATGCACAAGATAATACAAAGGATA
 I  I  A  Q  L  L  F  L  D  A  Q  D  N  T  K  D  I

       110                 130                 150
TTTACCTTTATGTCAATACACCAGGTGGTTCAGTATCGGCTGGACTTGCT
  Y  L  Y  V  N  T  P  G  G  S  V  S  A  G  L  A

                    170                 190
ATTGTGGACACCATGAACTTCATTAAATCGGACGTACAGACGATTGTTAT
I  V  D  T  M  N  F  I  K  S  D  V  Q  T  I  V  M

       210                 230                 250
GGGGATGGCTGCTTCGATGGGAACCATTATTGCTTCAAGTGGTGCTAAAG
 G  M  A  A  S  M  G  T  I  I  A  S  S  G  A  K  G

                    270
GAAAACGTTTTATGTTACCGAATGCAGAATATATGATC
  K  R  F  M  L  P  N  A  E  Y  M  I
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